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Are the correlation results of HPV positive cases with cervical smear always consistent?

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ABSTRACT:

Aim: Cervical cancer is a type of genital cancer which come second after breast cancer in women and may cause death. However, it can be prevented with screening tests by ensuring early diagnosis. Most of cervical cancers (99.7%) are associated with Human Papilloma Virus (HPV) and it is known that HPV must be present for the generation of cervical cancer. Thus, 70% of the patients have been found to be positive for HPV-16 and HPV-18. Both the association with cervical cancer and HPV and data related to development of cancer and dysplasia was researched retrospectively in this study. It was aimed to analyze and use the results by examine if the smear results of the patients and the HPV results are compatible in all HPV positive cases.

Material and Method: 1050 patients who were examined by gynecologist and taken Pap smear test at the end of the examination during July 2020 and March 2022, were included in our study. In all cases the Pap test was re-evaluated by the same pathologist using the 2014 Bethesda System. High-risk HPV (HR HPV) DNA (HPV types 16,18,31,33,35,39,45,51,52,56,58,59,66,68) tests results, applied to the patients at an external center, were reviewed. Results of patients diagnosed as LSIL, HSIL and cervical cancer by Pap smear test were evaluated again beside HPV DNA analyses and their clinical information. Chi-square test was used for statistical analysis. $p<0.05$ values were considered statistically significant.

Results: Of 1050 patients, 139 had LSIL, 170 had HSIL, and 112 had cervical cancer. The highest incidence of LSIL, HSIL and cancer was observed in the 30-39 age group, while the rate of these diseases was lower in the 50-59 age group compared to other groups. In addition, all patients with SIL and cervical cancer had smoking history. HR HPV DNA test was positive in 240 of 1050 patients. 56 patients diagnosed as LSIL and 89 patients diagnosed as HSIL by Pap smear had positive HR HPV DNA test results. HR HPV DNA positivity was reported in 74 of 112 patients with cervical cancer. In 21 patients who had normal smear test were detected HR HPV DNA positivity. 17 of these patients were in the 30-39 age group and 4 of them were in the 40-49 age group.

Conclusions: Because of all 112 patients with cancer were not HR HPV positive and the smear results of 21 HR HPV positive patients were normal, our study serves an example for studies to show that the results of smear and HPV DNA in cervical cancer are not always compatible.

Keywords: Cervix, cancer, HPV, screening

INTRODUCTION

Cervical cancer is ranked as the 4th most cancer among women worldwide. Its incidence and mortality have been gradually decreasing due to the development of early diagnosis and treatment methods and becoming widespread of cytological screening programs (1-5).

Pap smear test, which is used in early diagnosis, has high sensitivity and is easy to apply. It is low-cost and innocuous test, which also reduces the treatment burden, morbidity and mortality. Studies to prevent cervical cancer by using different screening methods are gaining importance in worldwide. Screening programs and strategies vary among countries (6-8,9).

In USA screening is started within the first three years following the age of onset of sexual intercourse or at the age of 21 at the latest. The screening begins at the age of 20 in Germany, for UK this age is 25 years. Screening is maintained until the age of 70 years. In Finland, which can be given as a more successful example, screening begins at the age of 30 and ceases at the age of 60 (9,10-12). Considering the conditions of our country, the achievable target is population-based screening, which starts at the age of 30 and ends at the age of 65 in women. Studies are going on to develop cervical cancer screening programs with various methods such as HPV DNA screening and to spread the screenings to the general population (13,14).

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The HPV DNA testing is the most objective and reproducible one among the current cervical screening tests. Evaluation of cervical cytology and simultaneous HPV DNA analysis is named as co-test. It is the most accepted screening method in women over the age of 30 (15,16).

Primary HPV DNA screening provides earlier detection of cervical lesions compared to cytology, but its effect on treatment options and adverse obstetric outcomes has not been comprehensively studied (16-18). It is expected to reduce both the risk of cancer and potential complications associated with cervical cancer screening by starting HPV DNA screening at the age of 20 (18,19).

MATERIAL AND METHOD

Research Design and Case Selection

This descriptive, retrospective study was conducted after the approval of the Kırıkkale University Non-invasive Clinical Researches Ethics Committee (Date: 29.06.2022, Decision No: 2022.06.33). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. 1050 of 2410 patients between the ages of 20 and 59 applied in the period of July 2020 and March 2022 to the Department of Obstetrics and Gynecology of Kırıkkale University Faculty of Medicine with complaints of abnormal vaginal bleeding and abdominal pain were selected as study group. Patients were divided into four age groups as follows: 20-29, 30-39, 40-49 and 50-59. Medical records of patients was searched and for each age group number of pregnancy and parity and whether they smoked were investigated.

Cases whose gynecological examinations and smear test sampling were performed were included in the study.

In addition, although not considered as a definite risk factors for cervical cancer, blood pressure measurement values in the file information and Hemogram and blood cholesterol levels measurements in biochemical analyzes were evaluated and large data sets were created.

In each age group, according to the results of the smear test the number of patients diagnosed with Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and cervical cancer was determined, and the cases that were positive and negative in the HPV DNA test were analyzed. The data of the selected patients for the analysis and the results were checked by the pathologist. Statistical analyzes and calculations were performed by means of IBM SPSS Statistics 21.0 program (IBM Corp. Released 2012. IBM SPSS Statisticsfor Windows, Version 21.0. Armonk, NY: IBM Corp.). Categorical variables were compared with the Chi-Square Test. A p-value below 0.05 were considered statistically significant.

Analysis of Smear and HR HPV DNA

For cytological analysis, the previously diagnosed cases taken with the conventional method and stained by applying normal Pap test staining procedures were removed from the archive and re-evaluated and interpreted based on the 2014 Bethesda System by the same pathologist. All datas were noted by forming groups as inflammation, LSIL, HSIL and cancer. By reviewing the tests for HPV DNA, two groups were recorded as positive and negative.

In high-risk HPV DNA (HR HPV DNA) analyzes, measurements were made for HPV types 16,18,31,33 ,35,39,45,51,52,56,58,59,66,68 and specific genotypic data were obtained with the qualitative multiplex assay method. Analyses were performed at Cancer Early Diagnosis Screening and Training Center.

Reassessment with physical examination findings was performed for the cases who had negative smear result with positive HPV DNA for any type. In addition, the cases diagnosed as LSIL, HSIL, and cancer without Hr HPV DNA were reanalyzed. There were no significant physical examination and biochemical analysis findings that would affect the results. The distribution of patients with or without cervical cancer was shown in **Figure 1**.

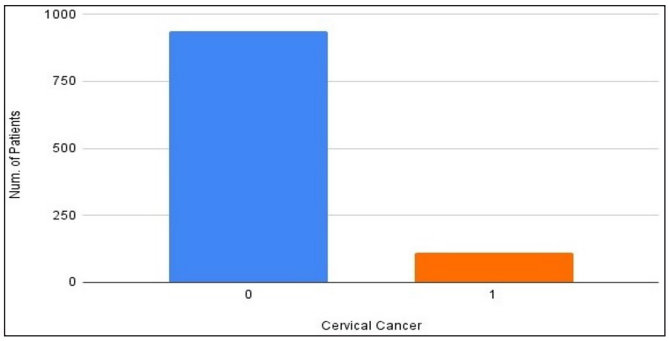

Figure 1: The distribution of cases according to having cancer (112 of 1050 had cervical cancer)

RESULTS

Considering the distribution of 1050 patients included in our study by age groups; There were 300 patients in the 20-29 age group, 360 patients in the 30-39 age group, 264 patients in the 40-49 age group and 126 patients in the 50-59 age group.

For the study group, the mean age was 37.23 ± 9.26 years. When the number of pregnancies of the patients was examined, the maximum number of pregnancies was five. However, the number of women who gave birth twice was the highest. In terms of distribution by age groups, it was highest in the 30-39 age group (n=162). In addition, the number of patients giving birth twice was higher in all age groups (n=364).

The least number of pregnancies was detected in the 20-29 age group. While there were 147 patients with one pregnancy, there was no patient with a fourth pregnancy in this group.

At the same time 20-29 age group had the lowest birth rate in the number of births. While 84 of 300 patients in this group gave birth twice, there was not found any patient being pregnant for four times.

Pregnancy and birth numbers by age groups were given in **Tables 1** and **2**.

Table 1. Number of pregnancies by age groups of patients

		Number of pregnancies					
Age range	Number (n)	0	1	2	3	4	5
20- 29	300	41	147	92	20	-	-
30-39	360	10	60	162	109	17	2
40-49	264	4	27	102	98	32	1
50-59	126	2	2	16	53	51	2

Table 2. Number of births by age groups of patients

		Number of birth				
Age range	Number (n)	0	1	2	3	4
20-29	300	41	145	84	13	-
30-39	360	10	60	162	109	17
40-49	264	4	27	102	96	30
50-59	126	2	2	16	50	51

There did not seen birth in any of the women who were pregnant for five times.

In addition, in cases where pregnancies in other age groups did not end with delivery and in some inconsistencies in the number of pregnancies and births, certain information about the possible termination of these pregnancies before delivery could not be reached.

Patients diagnosed as LSIL and HSIL in Pap test and HPV DNA results are shown in Tables 3 and 4 in **Figures 2** and **3**).

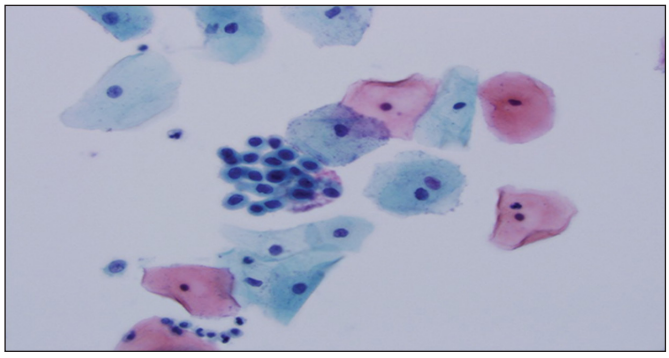

Figure 2: Low Grade Squamous Intraepithelial Lesion conventional Pap test (PAP x20)

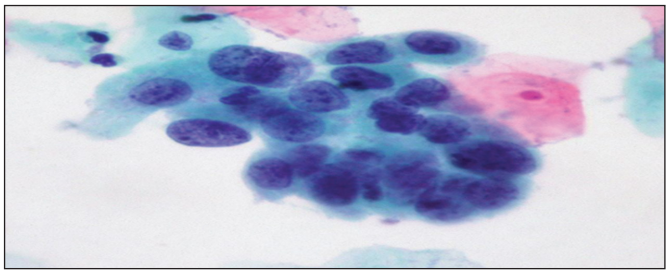

Figure 3: High Grade Squamous Intraepithelial Lesion conventional Pap test (PAP x40)

LSIL was detected in 139 patients. While the highest incidence of LSIL was detecetd in the 30-39 age group (n=47) the lowest incidence was detected in the 50-59 age group (n=22). HSIL was seen in 170 patients. HSIL was observed in 62 patients in the 30-39 age group at most (**Table 3** and **4**).

Table 3. Patients diagnosed with LSIL in Pap test and HPV DNA positivity rates

LSIL (*HPV DNA positive cases) p= 0.704			
Age range	Number n (%)	Pap test LSIL(+) HPV DNA(+) (n- %)*	Pap test LSIL (-) n
20-29	300 (31.50%)	34 (18-52.9%)*	266
30-39	360 (28.5%)	47 (20-42.5%)*	313
40-49	264 (25.1%)	36 (10-27.7%)*	228
50-59	126 (12%)	22 (8-36.3%)*	104

The rates and numbers given in black are calculated for patients diagnosed with LSIL in Pap test and HPV DNA positivity number and rates are given in red.

Table 4. Patients diagnosed with HSIL in Pap test and HPV DNA positivity rates

HSIL (*HPV DNA positive cases) p= 0.376			
Age range	Number n (%)	Pap test HSIL (+) HPV DNA (+) (n-%) *	Pap test HSIL (-) n
20-29	300 (31.50%)	38 (30-78.9%)*	262
30-39	360 (28.5%)	62 (35-56.4%)*	298
40-49	264 (25.1%)	50 (18-36%)*	214
50-60	126 (12%)	50 (18-36%)*	116

The rates and numbers given in black are calculated for patients diagnosed with HSIL in Pap test and HPV DNA positivity number and rates are given in red.

Cervical cancer was found in 112 of 1050 patients according to smear test results. While the highest incidence for cancer was detected in 30-39 age group with 45 patients and the lowest incidence was seen in 50-59 age group with 16 patients (**Table 5**).

Table 5. Patients diagnosed with cervical cancer in Pap test and HPV DNA positivity rates

Cervical Cancer (*HPV DNA positive cases) p=0.047			
Age range	Number n (%)	Pap test cancer (+) HPV DNA (+) (n-%)*	Pap test cancer (-) n
20-29	300 (31.50%)	13 (10-7.69%)*	287
30-39	360 (28.5%)	45 (40-88.8%)*	315
40-49	264 (25.1%)	38 (20-52.63%)*	226
50-59	126 (12%)	16 (4-25%)*	110

The rates and numbers given in black are calculated for patients diagnosed with cervical cancer in Pap test and HPV DNA positivity number and rates are given in red.

Considering that smoking is a risk factor in cervical cancer, the number of patients had history of smoking was investigated and it was observed that 478 patients were smoker. It was determined that 139 patients diagnosed with LSIL, 170 patients diagnosed with HSIL and 112 patients diagnosed with cervical cancer were all smokers.

HPV DNA test results of all patients were grouped for each age groups as negative and positive. HPV DNA positivity was seen in 240 of 1050 patients. Distribution of positivity by age groups was as follows: 58 in 20-29, 112 in 30-39, 52 in 40-49 and 18 in 50-59.

In 56 of 139 patients diagnosed with LSIL by Pap test, in 89 of 170 patients diagnosed with HSIL, in 74 of 112 patients diagnosed with cancer; HR HPV test was positive in 219 patients in total. There was a significant relationship between cervical cancer age groups and HR HPV DNA positivity (p=0.047).

Pap test results were normal in the repeat controls of 21 patients with positive HR HPV DNA tests.

In addition, the HR HPV DNA test was found to be negative in 38 patients diagnosed with cervical cancer. The percentage values of the data are shown in Table.3.

HR HPV DNA test was negative in 810 patients in total. The distribution of HR HPV DNA negative patients by age group is as follows: 242 patients in the 20-29 age group, 148 patients in the 30-39 age group, 212 patients in the 40-49 age group and 108 patients in the 50-59 age group.

DISCUSSION

Cervical cancer is the second most common cancer in women after breast cancer, causing a woman death every two minutes in the world (1,2). There are more than 400,000 cases of cervical cancer in the world annually. Approximately 250,000 patients die from cervical cancer each year (2,4,6). It is most common in the 50-59 age group. It is known that dysplastic changes in the cervix begin at an earlier age, in the 30-39 age group. Developing technologies, changes in lifestyles, the younger population being freer and changing partners frequently, the prevalence of viral infections cause a decrease in the age of dysplasia in the cervix (5,7).

The role of HPV in etiology is becoming increasingly important. The rate of cancer development in women infected with the virus in the high-risk group is higher than in other groups. So, HR HPV DNA analyzes are also included in cancer screening programs along with pap test (9-11).

In the study of Clavel et al. (12) they investigated the risk of developing LSIL over time in normal HPV negative smears and the potential of HR HPV types to develop LSIL. They reported that it would be correct to use Pap smear test and HR HPV DNA analyzes together.

There can be cases who have normal Pap smear results with HR HPV DNA positive. Contrary to this, there may be cases who have lesion detected by colposcopy or Pap smear and not infected HR HPV. In the literature, there are studies in which HR HPV DNA negativity was detected mostly in postmenopausal women (13,16).

In our study, there were patients (21 patients) whose HR HPV DNA analyses were positive and pap test smear results were normal for years.

Zergeroğlu et al. (18) reported that it was not appropriate to establish a screening program by performing HPV analysis alone in their study on menopausal patients among 5180 patients. They also showed that correlating Pap test and HR HPV DNA was practical to increase sensitivity and specificity and to prevent false positive and false negative results.

Öztürk et al. (16) emphasized that cervical cancer may develop in HPV-negative cases in their study. In our study, 38 of 112 cases with cervical cancer were found to be HR HPV DNA negative.

In addition, it is a fact that Pap test intake inadequacies to mislead in screening results. Therefore, diagnostic options with different methods are needed. Hosono et al. (15) reported in their study that inadequate pap test screenings in Japanese women affected the results.

In our study, it is important that only 74 of 112 patients diagnosed as cervical cancer had a positive HR HPV DNA test, and that the results of 21 patients with positive HPV analyses were normal after regular Pap test and colposcopic analyses. These results show that Pap test and HR HPV DNA analysis are not always correlated.

Sharif performed DNA analysis with PCR test to detect HR HPV type, in patients with suspected cervical neoplasia in his study. He showed that neoplasia detection rates increased in cases which was correlated with Pap test results. In addition, in his study, he found that this rate increased even more in cases in which the presence of neoplasia was supported in the biopsy as a result of colposcopic analysis performed (20).

In the retrospective study Song et al. (21) they analyzed the records of 4117 women who were selected from 33,531 Korean women who were performed Pap test, HPV analysis, cervicography, and colposcopic biopsy. They found the prevalence of cervical intraepithelial neoplasia 2 (CIN 2) cases to be 10.8% and the rate of HR HPV positive cases to be 61.0% by Pap test. With the

application of cervicography in addition to Pap test for the detection of CIN 2 cases, this rate increased to 97.5%, while the HR HPV positivity rate was 93.7%. These results support that it is not appropriate to use only one test alone for the diagnosis of cervical neoplasia.

In another study, Song et al. (22) showed that when Pap test and HPV tests were used together, they achieved excellent performance in detecting CIN or cervical cancer, and the combined use of the tests significantly reduced false negative errors.

CONCLUSION

In our study, similar to these studies in the literature, we concluded that it is not appropriate to perform Pap test or HR HPV DNA analysis alone in the detection of cervical neoplasia. We suggest that it would be more accurate to evaluate HR HPV DNA analysis and Pap test together in the detection of cervical neoplasia.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of KKU Faculty of Medicine Hospital Clinical Research Non-invasive Clinical Researches Ethics Committee (Date: 29.06.2022, Decision No: 2022.06.33).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the manuscript, and they have approved the final version.

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